Faculty of Resource Science and Technology

Bioactivities, Phytochemicals and Pharmacophore Analysis of Nipa Palm (*Nypa fruticans* Wurmb) Sugar Extracts

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Signature

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ABSTRACT

Nipa palm (*Nypa fruticans* Wurmb) sugar is used as a sweetener for local people especially in Sarawak. It was processed by using nipa palm sap as a raw material as it was cooked for several hours until it became brown syrup. Nipa palm sugar or widely known as gula apong. Nipa palm (*N. fruticans* Wurmb*)* sugar was extracted by using different solvents which were methanol (MeOH), ethanol (EtOH), water (H_2O), ethyl acetate (EtOAc) and hexane (Hex). All of the extracts were used to determine the moisture content, ash content, pH of the nipa palm sugar. Phytochemical screening was conducted in order to know the presence of the active compounds in the extracts. MeOH, EtOH and H_2O extracts were tested positive for the presence of saponins, tannins, terpenoids, flavonoids, phenolic compounds, reducing sugar and non-reducing sugar. The antioxidant activity of extracts was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and the results showed that nipa palm sugar extracts possessed antioxidant properties which were EtOH (IC_{50} = 200.6 µg/mL), MeOH (IC_{50} = 208.3 µg/mL), H_2O (IC_{50} = 569.8 µg/mL), EtOAc (IC_{50} = 1336.0 µg/mL) and Hex (IC_{50} = 4917.0 µg/mL). Alpha-amylase inhibition assay was performed by comparing with the acarbose and the results for the extracts were EtOH (IC_{50} = 109.40 µg/mL), MeOH (IC_{50} = 124.20 µg/mL) and H_2O (IC_{50} = 545.10 µg/mL). The antioxidant activity and alpha-amylase inhibition assay resulted that EtOH extract showed high inhibition for both assay. All of the extracts showed the low toxicity level against the brine shrimp (*Artemia salina*). The LigandScout 4.3 software based on Computer-Aided Drug Design (CADD) was used to identify the value of the nipa palm sugar in the pharmaceutical industry. Based on the ligand-based pharmacophore analysis, arginine showed the highest fit-value compared to other chemical constituents in nipa palm sugar, which is 89.42, with the interactions of 4 HBA, 4 HBD and 1 PI. Structure-based

pharmacophore modelling was generated based on a 3D crystal structure of human pancreatic alpha-amylase which had been retrieved from Protein Data Bank (PDB code: 1B2Y) and resulted that arginine compound of nipa palm sugar had the highest score for alignment score compared to other test sets which is 106.41 and it possessed two types of interactions which were HBA (ARG195A and HOH930A) and HBD (ASP300A, GLY233A, HIS299A and HOH610A). Thus, it was concluded that nipa palm sugar compounds showed that its compounds possess a great potential for inhibiting alpha-amylase.

Keywords: *Nypa fruticans* Wurmb, DPPH, alpha-amylase, CADD, ligand-based pharmacophore analysis

Bioaktiviti, Fitokimia dan Analisis Farmakafor terhadap Ekstrak Gula Pokok Nipah (*Nypa fruticans Wurmb*)

ABSTRAK

Gula nipah (Nypa fruticans Wurmb*) digunakan sebagai pemanis oleh orang tempatan terutamanya di Sarawak. Gula nipah diproses dengan menggunakan air nira sebagai bahan mentah, ia dimasak selama beberapa jam sehingga menjadi warna coklat dalam keadaan sirap. Gula nipah juga dikenali sebagai gula apong. Gula nipah (*Nypa fruticans Wurmb*) diekstrak dengan menggunakan pelbagai pelarut iaitu metanol (MeOH), etanol (EtOH), air suling (H_2O), etil asetat (EtOAc) dan juga heksana (Hex). Kesemua ekstrak digunakan untuk menentukan tahap kelembapan, kadar kandungan abu dan pH gula nipah. Saringan fitokimia telah dijalankan bagi mengetahui kewujudan kompaun aktif yang berada di dalam ektsrak. MeOH, EtOH dan H_2O ekstrak didapati positif untuk kewujudan kompaun saponin, tanin, terpenoid, flavonoid, kompaun fenolik, kekurangan gula dan juga tidak mengurangkan gula. Tindakan antioksidan juga telah dinilai menggunakan 2,2-diphenyl-1-picrylhydrazyl (DPPH) untuk mengikis aktiviti radikal bebas dan keputusan menunjukkan bahawa ekstrak gula nipah mempunyai antioksidan yang mana ekstrak EtOH (IC_{50} = 200.6 µg/mL), MeOH (IC_{50} = 208.3 µg/mL), H_2O (IC_{50} = 569.8 µg/mL), EtOAc (IC_{50} = 1336.0 µg/mL) da Hex (IC_{50} = 4917.0 µg/mL). Aktiviti perencatan enzim amilase telah dilaksanakan dengan membandingkan dengan acarbose dan keputusan untuk ekstrak adalah EtOH (IC_{50} = 109.40 µg/mL), MeOH (IC_{50} = 124.20 µg/mL) and H_2O (IC_{50} = 545.10 µg/mL). Aktiviti antioksidan dan perencatan enzim amilase menunjukkan bahawa ekstrak EtOH memberi perencatan yang tinggi untuk kedua-dua aktiviti tersebut. Kesemua ekstrak menunjukkan kadar toksik yang rendah terhadap udang air garam (*Artemia salina*). Perisian LigandScout 4.3 berdasarkan CADD telah digunakan untuk mengenalpasti nilai gula nipah dalam bidang*

farmasi. Berdasarkan analisi ligan farmakofor, arginin menunjukkan nilai yang paling tertinggi berbanding dengan komponen kimia yang lain di gula nipah, iaitu pada nilai 89.42, dengan interaksi 4 HBA, 4 HBD dan 1 PI. Pemodelan farmakofor berasaskan struktur telah dihasilkan berdasarkan struktur kristal 3D alpha-amilase pancreas manusia yang diambil daripada Bank Data Protein (kod PDB: 1B2Y) dan terhasil bahawa sebatian arginin gula apong mempunyai skor tertinggi untuk skor penjajaran berbanding dengan set ujian yang lain iaitu dengan skor 106.41 dan ia mempunyai dua jenis interaksi iaitu HBA (ARG195A dan HOH930A) dan HBD (ASP300A, GLY233A, HIS299A dan HOH610A). Dengan demikian, ia disimpulkan bahawa sebatian dalam gula apong menunjukkan bahawa sebatiannya memiliki potensi yang besar untuk merencatkan alpha-amilase.

Kata kunci: Nypa fruticans Wurmb, *DPPH, enzim amilase, CADD, analisi ligan farmakofor*

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LIST OF ABBREVIATIONS

AR	Aromatic
CADD	Computer Aided Drug Design
$CHCl_3$	Chloroform
DMSO	Dimethyl sulfoxide
DNS	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picry-hydrazyl
EtOAc	Ethyl acetate
EtOH	Ethanol
$FeCl_3$	Iron (III) chloride
H_2SO_4	Sulphuric acid
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HEX	Hexane
HY	Hydrophobic
LC_{50}	Lethal Concentration 50
MeOH	Methanol
NH_3	Ammonia
nm	Nanometre
PI	Positive Ionisable
SBDD	Structure Based Drug Design
UV-Vis	Ultraviolet–visible
μL	Microliter

CHAPTER 1

INTRODUCTION

1.1 Study Background

One of the most common and widely distributed in the mangrove forests of Southeast Asia is Nipa Palm (*Nypa fruticans* Wurmb). *N. fruticans* Wurmb found in the upstream estuarine zone which forms extensive stands along brackish to tidal freshwater creeks and rivers (Tamunaidu & Saka, 2011). It grows really fast especially in freshwater. *N. fruticans* Wurmb belong to the family of Arecaceae (Prasad et al., 2013). According to Duke (2006), the generic name of Nypa is derivative of "Nipah" which is the native name that was used in the Philippines and the Moluccas. "Fruticans' ' is a Latin word for shrubby which refers to its stem less appearance. *N. fruticans* Wurmb habitat is unique as it does not compete with food crops and its growth is sustainable. In addition, it also lacks an upright stem, which is one of the characteristic features for the palms.

The nipa palm sap is a good source of sugar and it is used for making sweets (nipa palm sugar), beverages, vinegar and alcohol (Joshi et al., 2006). The nipa palm sap was harvested from inflorescence (flower cluster) by making a cut on the inflorescence. The nipa palm sap was flown into a bamboo container which had been used by villagers in order to collect nipa palm sap. The nipa palm sap was boiled until the colour of the nipa palm sugar turned to brown syrup (Apriyantono et al., 2002). In the Southeast and South Asian regions such as Malaysia, Indonesia. Thailand, Philippines and India, nipa palm sugar has been used as a traditional and an alternative sweetener as the nipa palm (*Nypa fruticans* Wurmb) are found in abundance (Saputro et al., 2009).

In this study, the nipa palm (*N. fruticans* Wurmb) sugar extracts was analysed to determine the moisture content, ash content and pH. Hence, the phytochemical analysis was conducted in order to detect the presence of the saponins, tannins, phlobatannins, phenolic, terpenoids, reducing sugar and non-reducing sugar compounds in nipa palm sugar. Hence, the biological assay was conducted such as DPPH radical scavenging activity, alpha-amylase inhibition activity and brine shrimp lethality assay against the nipa palm sugar extracts.

According to Kanatt et al. (2007), the antioxidants from plant sources have been an increasing concern to the consumers since synthetic antioxidants such as butylated hydroxytoluene (BHT) have restricted usage in foods, because of the carcinogenic activity. Hence, the epidemiological studies have shown that frequent consumption of fruits and vegetables high in natural antioxidants can lower the incidence of certain types of cancer, cardiovascular diseases, and diabetes (Zambonin et al., 2012). These beneficial effects are related to bioactive compounds like phenolic acids, flavonoids, anthocyanin and carotenoids possessing antioxidant activity (Gordon et al., 2012).

Computer-Aided Drug Design (CADD) covers a broad range of applications spanning the drug discovery pipeline although these are highly clustered in the early phase. The main purpose of using CADD is to speed up and rationalize the drug design process while reducing costs (Taff et al., 2014). Hence, CADD is fast and automatic compared to traditional drug screening.

A pharmacophore model can be established either in a structure-based pharmacophore analysis by probing possible interaction points between the macromolecular target and ligands, or in a ligand-based pharmacophore analysis by superimposed a set of active molecules and extracted the common chemical features that are essential for their

bioactivity (Yang, 2010). The pharmacophore modelling was applied using LigandScout 4.3 software. LigandScout 4.3 software is an emerging and evolving modern computer science that provides an opportunity for researchers to go through a vast volume of data to identify whether a particular compound is suitable for pharmaceutical usage (Lallier, 2014). Pharmacophore modelling helps a lot in the detection of 2D and 3D molecules by identifying several key elements in the components of the sample (Sheridan & Kearsley, 2002). According to Qing et al. (2014), the 3D pharmacophore analysis was able to describe the action of a series of ligands, which is one of the computational chemistry's most important contributions to drug discovery. In addition, the chemical features of the pharmacophore model could be hydrogen-bond donor (HBD), hydrogen-bond acceptor (HBA), hydrophobic (HY), positive ionisable (PI) area, negative ionisable (NI) area and aromatic (Ar) (Salemme, 2001).

1.2 Problem Statement

The existing studies of other parts of *N. fruticans* Wurmb such as leaves, unripe endosperm, ripe endosperm, fruit husk had been mainly focused on antioxidant properties (Aziz & Jack, 2005; Prasad et al., 2013). Nipa palm sugar has been used as an alternative sweetener by local people to make desserts and sweets, and it might cause disease to the human being which is diabetes mellitus. Diabetes mellitus is a metabolic disorder that is caused by a defect in insulin secretion and insulin action (Bastaki, 2005). In this study, nipa palm (*N. fruticans* Wurmb) sugar extracts were analysed to determine its chemical constituents whether it possesses the ability to inhibit alpha-amylase. Other than that, nipa palm sugar compounds also were analysed by using computer aided drug design (CADD)

which are ligand-based pharmacophore model and structure-based pharmacophore model. CADD is a computational method which can discover, improve, learn drugs and any other related biologically active molecules (Zhang et al., 2017). In addition, the main target of CADD is to predict whether a given molecule will bind to a specific target (Yang, 2010). It helps to determine the compound which possesses a potential to be alpha-amylase inhibition. CADD was used due to its higher accuracy of results, faster and inexpensive cost compared to traditional methods. Thus, the research about the antidiabetic properties which is by inhibiting alpha-amylase of nipa palm sugar extracts could increase the medicinal value of the nipa palm tree.

1.3 Objectives

The objectives of this research are:

i. To determine the moisture content, ash content and pH of the nipa palm sugar extracts.

ii. To quantify the phytochemical constituents in nipa palm sugar extracts.

iii. To evaluate the antioxidant, alpha-amylase inhibition and toxicity activities of nipa palm sugar extracts.

iv. To analyse the antidiabetic properties of nipa palm sugar extracts by using pharmacophore modeling.

CHAPTER 2

LITERATURE REVIEW

2.1 *Nypa fruticans* Wurmb

2.1.1 Plant Profile

Nypa fruticans Wurmb (Figure 2.1) belong to the family of Arecaceae and grow abundantly in river estuaries and water environments where salt and freshwater mingle (Tamunaidu & Saka, 2011). Arecaceae consists of 181 genera and 2600 species and majority of them live in subtropical and tropical climates (Christenhusz & Byng, 2016). There are six major subfamilies of the Arecaceae which are Calamoideae, Coryphoideae, Nypoideae, Arecoideae, Phytelephantoideae and Ceroxyloideae (Dransfield, 2015). Hence, *N. fruticans* Wurmb belongs to the Nypoideae subfamily. Some of the significant species in the Arecaceae family are shrubs, tropical climbers and trees commonly known as palm trees.

Figure 2.1: *Nypa fruticans* Wurmb's tree

N. fruticans Wurmb which also being called as mangrove plants as it grows in a mangrove environment. According to Ebana et al. (2015), the habitat of *N. fruticans* Wurmb is considered as unique as it does not need to compete with food sources and its growth is also sustainable. Table 2.1 shows the taxonomy of *N. fruticans* Wurmb.

Table 2.1: The taxonomy of *N. fruticans* Wurmb (CABI, 2006)

Rank	Scientific Name
Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Arecales
Family	Arecaceae
Genus	*Nypa*
Species	*Nypa fruticans*

2.1.2 Vernacular Names of *Nypa fruticans* Wurmb

Nypa fruticans Wurmb had many vernacular names because every country had their own names. Therefore, Table 2.2 shows the vernacular names for *Nypa fruticans* Wurmb.

Table 2.2: The vernacular names of *Nypa fruticans* Wurmb (Baja-Lapis et al., 2004)

Countries	Vernacular names
Thailand	chak, at-ta
Vietnam	dua la, dua muoc
Myanmar	dani
Singapore	atap palm
Indonesia	nipah, buyuk, bobo
Malaysia	nipah
Papua New Guinea	biri-biri